



THE EFFECT OF INDUCED POLYPLOIDY ON THE FLAVONOLS OF *PETUNIA* 'MITCHELL'

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Abstract—In the flavonol biosynthetic pathway of *Petunia* 'Mitchell', polyploidy has a differential effect increasing the relative concentration of the major metabolite quercetin-3-sophoroside and decreasing the relative concentration of the minor metabolite quercetin-3,7-diglucoside. It has no effect on the total concentration or on the concentration of the other four minor flavonols.

INTRODUCTION

Naturally occurring polyploid cytotypes have been used to examine the effects of autopolyploidy on changes in the pool of secondary metabolites. In four different polyploid cytotypes (tetraploid, hexaploid, octaploid and decaploid) of *Atriplex confertifolia* L. Orach., the concentration of chlorophyll in the bundle sheath cells was directly proportional to the ploidy level [1]. The tetraploid cytotype had nearly twice the concentration of chlorophyll as the diploid cytotype. Such studies with naturally occurring polyploids could be misleading, for a polyploid cytotype and its diploid progenitor would be expected to accumulate a different spectra of mutations over time. These mutations could influence metabolite pools.

Very few workers have examined the accumulation of secondary metabolites in artificially induced autopolyploids. In one report, a 40% increase in total carotenoid was found in seed of colchicine-induced tetraploid *Zea mays* L. as compared with seed from the diploid progenitor [2]. Such studies with artificially induced polyploids are very important in understanding the effects of gene dosage on secondary metabolite pools, because of the absence of confounding mutations that could accumulate in naturally occurring polyploids.

There have been several reports that colchicine, besides inducing polyploidy, can also induce other chromosomal mutations in ancient allotetraploid plants [3]. These other mutations are found in the diploid progeny of treated plants and are the result of chromosome substitution. Colchicine can interfere with chromosome pairing during meiosis, resulting in chromosome substitutions [4]. This additional effect of colchicine could be avoided by treating immature plants (i.e. non-flowering) with colchicine.

This paper compares the floral flavonols found in artificially induced diploid, tetraploid and octaploid cytotypes of *Petunia* 'Mitchell' with the haploid progenitor. *Petunia* 'Mitchell' is a haploid plant derived from anther culture of a selected seedling from the backcross population of (*P. axillaris*, Juss. \times *P. hybrida*, Vilm. 'Rose du Ciel') \times *P. axillaris* [5]. The cultivated petunia (*P. hybrida*) was derived from *P. axillaris*.

RESULTS AND DISCUSSION

In *Petunia* 'Mitchell', an increase in the ploidy caused an increase in the width of the leaves and the length, width and the volume of palisade cells (Table 1). The morphology of the haploid, diploid and tetraploid plants was very similar except in size. Octaploid plants produced both deformed leaves and flower buds which aborted before opening; therefore, the floral flavonols from the octaploid cytotype could not be determined.

No qualitative differences in the flavonol profiles of the haploid, diploid or tetraploid cytotypes were observed (Table 2). Each cytotype contained every flavonol, and there were no flavonols specific to a given cytotype. Qualitative differences between artificially induced tetraploids and their diploid progenitors have been reported; however, these reports are in a minority [6]. In the diploid cytotype of *Briza media*, L. Per., C-glycosyl derivatives of apigenin accumulate, while in the tetraploid cytotype, C-glycosyl derivatives of luteolin accumulate [7]. Less dramatic differences were observed in the flavonoid profile of tetraploid and diploid cytotypes of *Phlox drummondii*, Hook [8]. The flavonoid profile of tetraploids included several new minor flavonoids that did not appear in the diploid

Table 1. Morphology of the haploid, diploid, tetraploid and octaploid cytotypes of *Petunia* 'Mitchell'

Ploidy	Leaf width	Palisade		
		Length	Width	Volume
Haploid	92*	21	10	0.50
	(4)†	(5)	(3)	(0.04)
Diploid	197	42	18	1.36
	(2)	(7)	(3)	(0.06)
Tetraploid	251	62	24	3.57
	(10)	(8)	(4)	(0.13)
Octaploid	412	93	28	7.29
	(20)	(8)	(6)	(0.29)

Values are reported in either μm or $\times 10^{-8} \text{ cm}^3$ and are the mean of 5 measurements from 5 different plants.

*Mean.

†Standard deviation.

profiles. In addition, eight flavonoids that were present in the diploid profiles were absent in the tetraploid profiles.

We observed no quantitative differences in the total concentration of floral flavonols in the various cytotypes of *Petunia* 'Mitchell' (Table 2). However, quantitative differences were found in concentration of specific flavonols. As the ploidy level increased, the percentage of quercetin-3-sophoroside within the total flavonol pool increased, while the relative concentration of quercetin-3,7-diglucoside decreased. Spearman's Rank Correlation Coefficient between ploidy and the concentration of quercetin-3-sophoroside was 0.851 with a t-test value of 5.13. Similarly, the coefficient of correlation between ploidy and the concentration of quercetin-3,7-diglucoside was -0.843 with a t-test value of -4.95 . Both correlations were acceptable at the 99.9% probability level.

There are several reports describing quantitative differences between cytotypes after polyploidy induction [6]. In tetraploid *Z. mays*, there was a five-fold increase in the amount of carotenoids per cell, which translated into a 2.5-fold increase per gene as compared to the diploid progenitor [2]. Similarly, there was a two-fold increase in alcohol dehydrogenase activity in

tetraploid *P. drummondii* as compared to the diploid cytotype (9). However, in *P. drummondii* ssp. *mcallisteri*, there were no observed differences in alcohol dehydrogenase activity between the tetraploid and its diploid cytotype [9]. The activity of esterase in induced tetraploids and their diploid progenitors was compared for 29 different species [10]. In 17 of 29 species examined the level of esterase was found to be higher in the tetraploid. Artificially induced autopolyploidy can both quantitatively and qualitatively affect the pool of secondary metabolites. In *Petunia* 'Mitchell', induced polyploidy had a quantitative effect on only two specific flavonols and not on the total flavonol pool.

EXPERIMENTAL

Plant material. A haploid *Petunia* 'Mitchell' plant was generously supplied by Dr Maureen Hanson of Cornell University, Ithaca, NY. This plant was maintained and propagated *in vitro* on medium containing Murashige-Skoog salts and vitamins, 20 g l^{-1} sucrose and 8 l^{-1} agar as previously described [11]. Cultures were maintained at 25° with 16 hr daylight of $260 \mu\text{mol}^{-1} \text{ m}^{-2}$ cool white fluorescent illumination.

Polyploidy induction. The diploid cytotype arose

Table 2. Analytical HPLC data for the flavonols of haploid, diploid and tetraploid cytotypes of *Petunia* 'Mitchell'

Ploidy	Flavonol						Total
	Q3	Q3 ²	Q7	Q3,7	Q3 ² ,7	Qc3 ² ,7	
Haploid	1.5*	73.6	7.9	14.0	1.5	1.5	1.7
	(0.1)†	(2.1)	(0.6)	(2.5)	(0.1)	(0.1)	(0.3)
Diploid	1.5	79.4	7.7	8.5	1.5	1.5	1.9
	(0.1)	(2.2)	(1.2)	(1.1)	(0.1)	(0.1)	(0.5)
Tetraploid	3.5	81.3	9.3	3.5	1.2	1.2	1.5
	(0.2)	(1.9)	(1.4)	(0.9)	(0.1)	(0.1)	(0.1)

Values are reported as the percentage of the total quercetin present and are the mean of 4 measurements. Total concn is in $\mu\text{g mg}^{-1}$ dry wt.

Abbreviations: Q3 = quercetin-3-glucoside, Q3² = quercetin-3-sophoroside, Q7 = quercetin-7-glucoside, Q3,7 = quercetin-3,7-diglucoside, 3²,7 = quercetin-3-sophoroside-7-glucoside, Qc3²,7 = quercetin-3-cafeoylsophoroside-7-glucoside.

*Mean from 4 different plants.

†Standard deviation.

spontaneously from haploid cytotypes cultured *in vitro*. This was a frequent event. Polyploidy was induced through *in vitro* colchicine treatment of non-flowering shoot tips. Shoot tips were transferred to medium containing 0.1 mg l^{-1} filter-sterilized colchicine. After 1 week, the treated tips were removed and placed on colchicine-free medium. Lateral shoots that developed were propagated and established in the greenhouse.

The ploidy level of greenhouse grown plants was determined both by chromosome counts of root tips and microspectrophotometry of leaf cells [11].

Flavonoid analysis. The flavonols were extracted from flowers and analysed by HPLC as previously described [12]. Fresh flowers were extracted in 50 ml 100% MeOH and the extracts evapd to dryness at 40° under red. pres. and redissolved in $50 \mu\text{l}$ 100% MeOH. The flavonols were resolved on a Bondapak C_{18} column using a 20 min linear gradient of 0–20% MeCN in 1% Et_3N at pH 3.0. The MeCN concn was held at 20% for an additional 20 min. The flow rate was 1 ml min^{-1} (ca 1500 psi) and the eluate monitored at 340 nm. For each cytotype, flowers from 4 different plants were analysed. From each plant, 5 flowers were independently extracted. The means and standard deviations were then determined.

REFERENCES

1. Warner, D. A. and Edwards, G. E. (1980) *Plant Physiol.* **91**, 1143.
2. Randolph, L. F. and Hand D. B. (1940) *J. Agric. Res.* **60**, 51.
3. Franzke, C. J. and Sanders, M. E. (1965) *Am. J. Botany* **52**, 211.
4. Bennett, M. D., Toledo, L. A. and Stern, H. (1979) *Chromosoma* **72**, 175.
5. Mitchell, A. Z., Hanson, M. R., Skvirsky, R. C. and Ausubel, F. M. (1980) *Z. Pflanzphysiol.* **100S**, 131.
6. Mears, J. A. (1980) *Polyploidy* (Lewis, W. H., ed), p. 77. Plenum Press, New York.
7. Murray, B. G. and Williams, C. A. (1976) *Biochem. Genet.* **14**, 897.
8. Levy, M. (1976) *Biochem. Syst. Ecol.* **4**, 249.
9. Levin, D. A., Torres, A. M. and Levy, M. (1979) *Biochem. Genet.* **17**, 35.
10. Nakai, Y. (1977) *Jpn J. Genet.* **52**, 171.
11. Kamo, K. K. and Griesbach, R. J. (1989) *Plant Sci.* **65**, 119.
12. Griesbach, R. J. and Asen, S. (1990) *Plant Sci.* **70**, 49.